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OSMOTIC REFLEXION COEFFICIENTS OF CAPILLARY WALLS TO LOW MOLECULAR WEIGHT HYDROPHILIC SOLUTES MEASURED IN SINGLE PERFUSED CAPILLARIES OF THE FROG MESENTERY

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SUMMARY

1. Individual capillaries of the transilluminated frog mesentery have been perfused with suspensions of human red cells in frog Ringer solution containing 1·0 g albumin 100 ml.$^{-1}$. The outer surface of the mesentery has been washed with normal frog Ringer solution and with frog Ringer solutions made hypertonic by addition of one of the following solutes: sodium chloride (100 m-mole.1.$^{-1}$); urea (100 m-mole.1.$^{-1}$); sucrose (20–50 m-mole.1.$^{-1}$); cyanocobalamin (8·5 m-mole.1.$^{-1}$). The temperature of the mesentery was between 14 and 16° C in all experiments.

2. With the mesentery superfused with normal Ringer, the filtration coefficient was determined from measurements of the rate of fluid filtration across the capillary wall, at a series of known capillary pressures (Michel, Mason, Curry & Tooke, 1974). Filtration coefficient varied from $0{\cdot}69 \times 10^{-3}$ to $4{\cdot}45 \times 10^{-3}$ μm.sec^{-1}.cm H_2O^{-1} with an average value of $1{\cdot}87 \times 10^{-3}$ μm.sec^{-1}.cm H_2O^{-1}.

3. When the superfusate was made hypertonic by the addition of a test solute, the osmotic reflexion coefficient (σ) of the capillary wall to test solute was calculated from the additional rate of filtration, the concentration of test solute in the superfusate and the filtration coefficient. Average values for σ were: sodium chloride, $0{\cdot}068 \pm 0{\cdot}03$ (three capillaries); urea, $0{\cdot}071 \mp 0{\cdot}015$ (four capillaries); sucrose, $0{\cdot}115 \mp 0{\cdot}023$ (seven capillaries); cyanocobalamin, $0{\cdot}100 \mp 0{\cdot}03$ (three capillaries).

4. In further experiments, the osmotic reflexion coefficients to sodium chloride, urea and sucrose were determined in the same capillary. Five technically acceptable experiments were carried out. Although there were

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differences in the value of σ between different capillaries, in any one capillary values of σ were of the same magnitude and there appeared to be no significant trend with the molecular size of the test solute.

5. Our findings are inconsistent with the hypothesis that there is a single pathway for water and small hydrophilic molecules across the capillary wall.

6. Our results may be interpreted in terms of an exclusive channel for water in parallel with a channel shared by both water and small hydrophilic molecules. It is suggested that the exclusive water channel may be the membranes and cytoplasm of the endothelial cells and the shared channel may be located in the intercellular junctions.

7. Our data suggest the exclusive water channel represents about 10% of the total filtration coefficient in frog mesenteric capillaries. The shared channel shows relatively little restriction to the molecules investigated. Estimates of the volume flow through the two channels are made for conditions where hydrostatic pressure differences and osmotic pressure differences are the driving forces.

INTRODUCTION

The experiments described in this paper were designed to investigate whether net water flow across the capillary wall is entirely restricted to channels available to small hydrophilic molecules or whether some water can cross the capillary wall by a route unavailable to solutes. To do this, we have measured the osmotic reflexion coefficients, σ, of the capillary wall to a series of small hydrophilic solutes. A measure of σ is a measure of the selectivity of a membrane to water and a test solute, for a semipermeable membrane $\sigma = 1$, for an unselective membrane $\sigma = 0$. If there is a single shared pathway for water and small hydrophilic molecules, the values of σ for these molecules should vary with molecular size in a manner predicted by the pore theory (Durbin, 1960; Solomon, 1968; Bean, 1972; Curry, 1974; Anderson & Malone, 1974). If in addition to the shared channel there is a channel impermeable to solute but available for net water flow, σ should not vary as predicted with molecular size and extrapolation of the data should suggest that σ would be greater than zero for solute molecules equal in size to water.

Although σ is defined under conditions of zero volume flow, it can be calculated from the osmotic flow across a membrane of known filtration coefficient (L_p). The total volume flow per unit area of membrane (J_v/A) is dependent upon differences in hydrostatic pressure (ΔP) and osmotic pressure ($\Delta\Pi$), i.e.

$$J_v/A = L_p \Delta P - L_p \sigma RT \Delta C, \quad (1)$$

whence

$$\sigma = \frac{L_p \Delta P - (J_v/A)}{L_p RT \Delta C}, \qquad (2)$$

where ΔC is the difference in solute concentration across the capillary wall.

Several attempts have been made to measure σ of capillary walls to small hydrophilic molecules (Vargas & Johnson, 1964; Taylor & Gaar, 1970; Perl, Chowdhury & Chinard, 1975). These experiments have been carried out on perfused organs where difficulties of estimating the trans-capillary concentration gradient and the appropriate osmotic flow render the results open to serious criticism (e.g. Pappenheimer, 1970 *b*). To minimize these objections we have attempted to measure σ in single perfused capillaries of the frog mesentery. These are continuous capillaries which can be individually perfused via a micropipette whilst the thin tissues of the mesentery are washed with superfusates of known composition.

Sucrose, urea, sodium chloride and cyanocobalamin were used as test solutes. In the first series of experiments σ was determined for only one test solute on each capillary investigated. In a later series, it was possible to determine values of σ for sodium chloride, urea and sucrose on the same capillary. The results have been described in two preliminary communications (Curry, Mason & Michel, 1974; Michel, Curry & Mason, 1975).

METHODS

Preparation. All experiments were carried out on mesenteric capillaries of *Rana temporaria*. The brains of these animals were pithed but the spinal cords were left intact. The abdominal cavity was opened and the mesentery carefully laid over a short Perspex pillar so that it could be trans-illuminated. The exposed tissue was kept moist and cool by washing its upper surface continuously with a superfusate of cooled Ringer solution which sometimes contained the test solute at a known concentration. A thermistor glued to the upper surface of the pillar allowed a continuous record to be made of the mesenteric temperature which was always in the range of 14–16° C.

Solutions. In all experiments except those described under the heading of 'internal sucrose', the perfusate was a frog Ringer solution containing crystalline bovine albumin at a concentration of 1·0 g.100 ml.$^{-1}$. The pH of the solution was measured with a glass electrode and adjusted to be in the range 7·30–7·60 by adding a few drops of N-NaOH. A small number of human red cells were suspended in the perfusate (haematocrit *ca.* 2 %) and acted as flow markers. The preparation of the red cell suspension has been described previously (Michel *et al.* 1974).

The superfusate for control measurements was a frog Ringer solution which, apart from containing no protein or red cells, was identical in all other respects to the perfusate. For the measurement of the osmotic reflexion coefficient the superfusate was a Ringer solution to which test solute had been added in one of the following concentrations (m-mole.l.$^{-1}$): sucrose, 20, 30, 50; urea, 100; sodium chloride, an additional 100; cyanocobalamin, 8·5. The normal Ringer solution had a composition (m-mole.l.$^{-1}$): NaCl, 111·1; KCl, 2·4; $MgSO_4.7H_2O$, 1·0; $CaCl_2$, 1·1; glucose, 5·5.

Micropipettes and procedure of cannulating and perfusing capillaries. The micropipettes, microrods used for micro-occlusion, the manometer system and the technique of perfusing single mesenteric capillaries with suspensions of red cells have been described previously (Michel, Baldwin & Levick, 1969; Michel *et al.* 1974).

Observation and recording red cell movements. The trans-illuminated mesentery was viewed using a Wild M5 dissecting microscope which possessed a trinocular fitting for simultaneous viewing and photomicrography. A prism fitted to the top of the camera tube projected the image of the field into a television camera. A second television camera recorded the scale of a digital clock and the face of an oscilloscope tube on which were recorded the temperature of the mesentery and the pressure in the manometer attached to the micropipette. A mixing box (ITC type MEA 5100) was used to combine the outputs from the two cameras and the composite picture together with a verbal commentary was recorded on $\frac{1}{2}$ in. tape using a Shibaden videotape recorder (SV–610K). The movements of the suspended red cells were measured by playing back the video recordings in slow motion and recording their position on stationary single frames (see Michel *et al.* 1974). Capillary radius was estimated from the mean of at least three separate measurements of capillary diameter made (directly from the television screen) at intervals of 50–80 μm along the length of the capillary.

Measurement of filtration coefficients of single capillaries. The filtration coefficient of perfused single capillaries was determined from a series of measurements of filtration rate at different capillary pressures (Method I of Michel *et al.* 1974). Experiments were carried out on straight capillaries all of which had an initial length greater than 1000 μm. The vessel was cannulated with a micropipette and perfused at a high flow rate at a series of different pressures. Initially, the vessel was occluded as far downstream as possible from the site of cannulation but as the experiment progressed the occlusion site was advanced towards the micropipette so as to prevent damaged areas of the capillary from influencing the results.

RESULTS

Measurements of reflexion coefficients to sodium chloride, urea, sucrose and cyanocobalamin

In all these experiments, a single capillary was cannulated and perfused with normal frog Ringer solution containing albumin (1·0 g.100 ml.$^{-1}$). The preparation was washed first with Ringer solution free of test solute and a control value of the filtration coefficient of the capillary was determined from a series of estimates of filtration rate at different capillary pressures. The superfusate was then changed to a Ringer solution containing the test solute and the measurements of filtration rate at different capillary pressures were repeated. The original superfusate free of test solute was then returned and the filtration coefficient was determined for a third time (second control). If the first and second control measurements of filtration coefficient differed significantly, the experiment was rejected. This happened in three experiments of this series. A larger number of experiments (more than fifty) were abandoned before the second control measurement could be made, most often owing to peristaltic contractions

of the intestine moving the mesentery. In some experiments, however, it was possible to repeat the estimates of filtration coefficient in the presence of test solute together with control measurements for a second and even a third time.

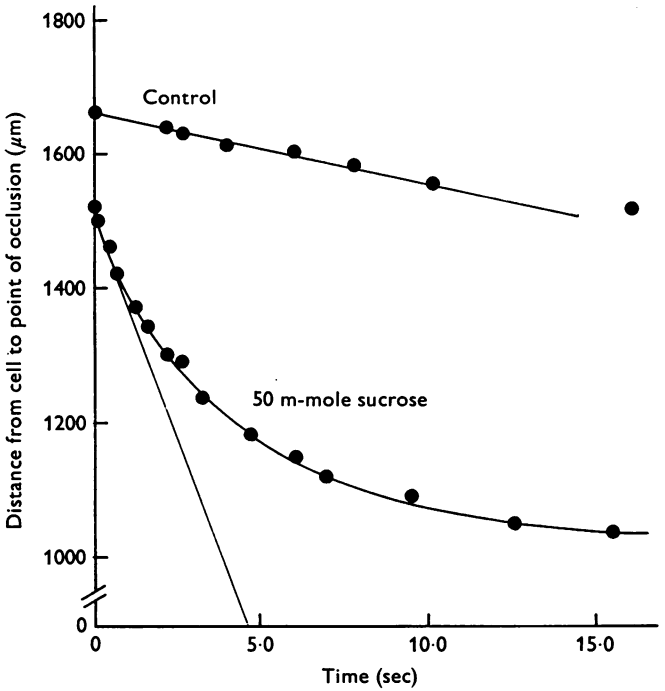

Fig. 1. The movement of red cells resulting from the net outflow of fluid across the walls of an occluded capillary. The capillary was occluded at zero time; the ordinate represents the distance in μm between the red cell and the point of occlusion. The capillary was perfused with frog Ringer containing 1·0 g albumin 100 ml.$^{-1}$ at 30 cm H_2O. Upper curve, tissue superfused with frog Ringer solution; lower curve, tissue superfused with frog Ringer solution to which sucrose has also been added at a concentration of 50 m-mole.l.$^{-1}$. The smooth curve and the initial tangents were fitted to the curve by eye.

Fig. 1 compares the movements of a red cell following micro-occlusion in the same capillary at the same hydrostatic pressure when the superfusate was free of test solute and when sucrose had been added to the superfusate in a concentration of 50 m-mole.l.$^{-1}$. When sucrose was present in the superfusate the initial rate of filtration was more than ten times greater than the control. The slopes of the lines drawn through the initial parts of the curves indicate the velocities used for calculating these initial filtration rates and it can be seen that measurements of red cell

position can be made frequently enough for these lines to be drawn accurately even when the red cell velocity is relatively high.

The results of an experiment where sucrose was again the test solute (this time at a concentration of 20 m-mole.l.$^{-1}$) are shown in Fig. 2 as the relationship between the initial filtration rate per unit area of capillary

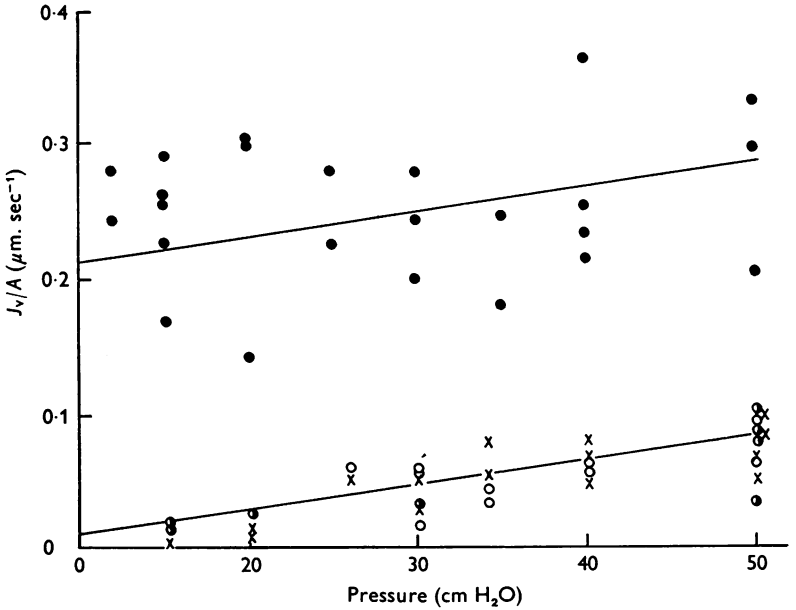

Fig. 2. The relationship between filtration rate per unit area of capillary wall (J_v/A) and capillary pressure in a capillary. The lower points represent data obtained when the tissue was superfused with Ringer solution. Three separate determinations of the filtration coefficient, measured as the slopes of the regression line through the points indicated by different symbols, were $1{\cdot}54 \times 10^{-3}$, $1{\cdot}89 \times 10^{-3}$, and $1{\cdot}89 \times 10^{-3}$ μm.sec^{-1}.cm H_2O^{-1}. The line shown has the slope of the mean filtration coefficient and cuts the abscissa at a pressure of 4 cm H_2O. The upper points are measurements of the initial filtration rate on the same capillary when the tissue was superfused with frog Ringer solution to which sucrose had been added to a concentration of 20 m-mole.l.$^{-1}$. The line drawn through these points is not a regression line but a line of slope equal to the mean filtration coefficient drawn through the mean value of this population. There is no suggestion that the filtration coefficient is increased by the hypertonic sucrose containing superfusate.

wall and the capillary pressure. The lower line is a regression line drawn through the values of filtration rate determined when sucrose was absent from the superfusate: its slope is equal to the filtration coefficient. Three sets of control measurements made before and after superfusion with the

sucrose containing solution are represented by the different symbols. The agreement between them indicates there was no change in the control filtration coefficient during the course of the experiment. The upper group of points represent measurements made when sucrose was present in the superfusate. These points show a conspicuous increase in the initial rate of filtration at all capillary pressures. Although the scatter of these points is greater than that of the control group, there is no tendency for the initial

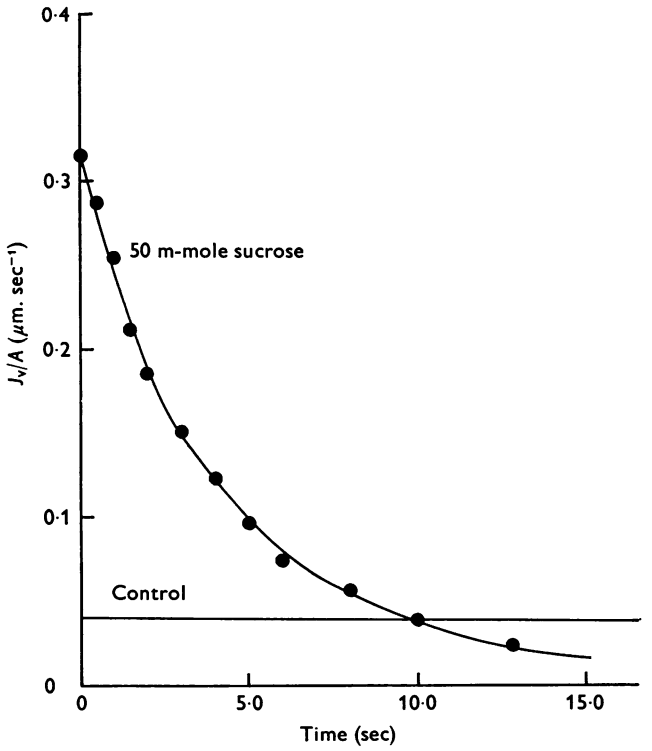

Fig. 3. The relationship between filtration rate per unit area of capillary wall and time for the data presented in Fig. 1. The points were obtained by drawing a series of tangents to the smooth curve shown in Fig. 1. The lower control curve is fixed at time zero by the initial slope through the control data and is drawn to decline very slightly with time as the albumin concentration is raised by fluid loss from the closed off capillary. The vertical distance between the two curves is $L_p \delta_s RT \Delta C_s$ where ΔC_s is the difference in sucrose concentration across the capillary wall.

rate of filtration to be more sensitive to changes in capillary pressure than it is under control conditions. This was a general finding of all the experiments reported in this paper and it was taken to indicate that the

filtration coefficient is not increased by the hypertonic solutions used. Additional evidence supporting this conclusion is provided by the rates of filtration observed when the contents of an occluded capillary are approaching equilibrium with the superfusate. Fig. 3 represents the data of Fig. 1 as the changes in filtration rate with time following micro-occlusion. Filtration rate per unit area of capillary wall is seen to fall below the control level by 10 sec. If the hypertonic superfusate had increased the filtration coefficient of the capillary, the filtration rate would be expected to be considerably raised above the control level after solute equilibration was complete. That filtration rate fell below the control value was a consistent finding with urea and sucrose as test solutes. The possible reasons for this are discussed below.

TABLE 1. Osmotic reflexion coefficients (σ) of single capillaries to various solutes

Solute	Molecular radius (nm)	Number of capillaries	σ* (S.E. of mean)	$L_p \times 10^3$ (μm.sec^{-1}.cm H_2O^{-1})
Sodium chloride	0·23	3	0·068 ($\mp$ 0·03)	2·79
Urea	0·26	4	0·071 ($\mp$ 0·015)	2·21
Sucrose	0·48	7	0·115 ($\mp$ 0·023)	2·62
Vitamin B_{12}	0·80	3	0·100 ($\mp$ 0·03)	2·14

* Between ten and thirty determinations of σ were made on each capillary investigated.

In an experiment such as that shown in Fig. 2, the osmotic reflexion coefficient was estimated for each determination of filtration rate made in the presence of test solute using eqn. (2). The numerator on the right hand side of eqn. (2) was taken as the difference between the initial filtration in the presence of test solute and the filtration rate read at the same capillary pressure from the regression line through the control data. The final value of σ for a particular capillary was taken as the arithmetical mean of these estimates. The results of all the experiments when σ for only one solute was investigated per capillary are presented in Table 1. These results suggest there is little increase in σ with increasing molecular size. There was, however, considerable variation between different capillaries in the values of σ for any one particular solute. Differences in filtration coefficient (Zweifach & Intaglietta, 1968; Michel *et al.* 1974) and permeability (Levick & Michel, 1973) between individual capillaries in a capillary bed have been reported before and the possibility that variations of this kind might be obscuring a relationship between σ and molecular size was investigated in a further set of experiments.

Measurements of the reflexion coefficients to sodium chloride, urea and sucrose in a single capillary

To investigate whether variations in the permeability properties of different capillaries was hiding a relationship between σ and molecular size, we attempted to measure the reflexion coefficients of sodium chloride, urea and sucrose in the same capillary. Six experiments were technically successful but one of these was later rejected when the analysis of the results revealed a change of filtration coefficient during the course of the experiment.

TABLE 2. Osmotic reflexion coefficients of single capillaries to different solutes

Capillary	Average $L_p \times 10^3$ (μm.sec^{-1}. cm H_2O^{-1})	Reflexion coefficient		
		Sodium chloride	Urea	Sucrose
A	0·69 (∓ 0·10)	0·200 (∓ 0·04)	0·220 (∓ 0·04)	0·285 (∓ 0·07)
B	0·98 (∓ 0·17)	0·064 (∓ 0·007)	0·024 (∓ 0·02)	0·091 (∓ 0·035)
C	2·40 (∓ 0·51)	0·102 (∓ 0·026)	0·131 (∓ 0·03)	0·114 (∓ 0·03)
D	1·45 (∓ 0·24)	0·113 (∓ 0·021)	0·169 (∓ 0·034)	0·129 (∓ 0·03)
E	1·54 (∓ 0·23)	0·152 (∓ 0·025)	0·168 (∓ 0·03)	0·132 (∓ 0·026)

* The results from capillary *F* were rejected as L_p changed during the course of the experiment. There was some uncertainty also about the absolute value of L_p in capillary *B* since poor illumination of the tissue considerably reduced the accuracy of the measurement of capillary radius.

Standard errors of the mean of L_p were calculated using each measurement of (J_v/A) and $(\Delta P - \Delta\Pi_p)$ in the absence of test solute. Standard errors of the mean of σ were calculated using each value of $\Delta\ (J_v/A)/(L_p RT\Delta C)$ in the presence of test solute.

The protocol of these experiments differed slightly from the earlier experiments. The filtration coefficient was determined by the measurement of filtration rate at two or three capillary pressures only. When test solute was added to the superfusate, several measurements of filtration rate were made at only one capillary pressure. Control measurements of filtration rate were always made in the absence of test solute between sets of measurements in the presence of different test solutes. The order in which the different test solutes were applied to the preparation was varied from experiment to experiment and in some capillaries it was possible to make more than one set of measurements for each of the test solutes.

The results of these experiments are summarized in Table 2. It is clear that although there are variations in the values of σ between different capillaries, in any one capillary there is no significant relationship between the values of σ and molecular size.

Experiments with internal sucrose

Three experiments were carried out in which sucrose was added to the perfusate but not to the superfusate. In these experiments, the capillary was perfused for only 2 sec before the measurement of net transcapillary flow so as to minimize the extracapillary sucrose concentration. After a measurement had been made, the pressure in the micropipette was lowered so that frog plasma (free of sucrose) was drawn into the vessel,

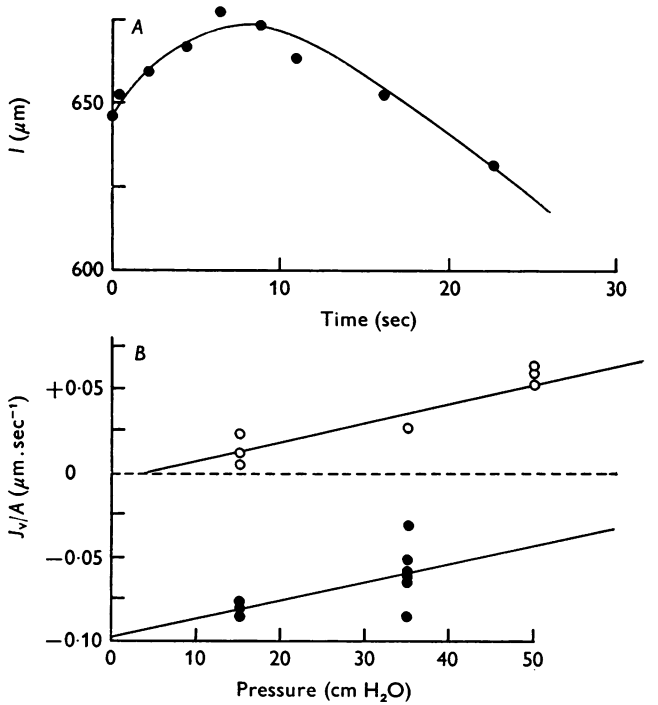

Fig. 4. Determination of the osmotic reflexion coefficient of a capillary to sucrose with sucrose added to the perfusate. *A*, the movements (*l*) of a red cell with time in a closed-off section of capillary in which the perfusate contains sucrose (30 m-mole.l.$^{-1}$) as well as the normal constituents of frog Ringer and 1·0 g albumin 100 ml.$^{-1}$. The superfusate was initially sucrose free. The pressure in the capillary is 35 cm H_2O. For the first 10 sec the cell moved away from the occlusion site indicating the movement of fluid from the tissues into the capillary but as the osmotic gradient dissipated the hydrostatic pressure difference across the capillary wall came to exceed the osmotic pressure difference and net flow changed direction. *B*, the relationship between filtration rate per unit area of capillary wall $(J_v/A)_0$ and capillary pressure when both perfusate and superfusate contained 30 m-mole.l.$^{-1}$ sucrose (upper curve) and when the sucrose was present only in the perfusate.

and a high flow of sucrose free superfusate was maintained. The pressure in the micropipette was then raised abruptly to a pre-set value and as soon as the perfusate had washed through the capillary, the vessel was occluded and fluid flow deduced from the red cell movements. When this protocol was followed, the red cells moved back towards the micropipette immediately after occlusion indicating that fluid was being absorbed into the capillary from the surrounding tissues. On some occasions at higher capillary pressures an early inward flow of fluid was observed to slow down and change direction to become a net outward flow as presumably the sucrose equilibrated across the capillary wall (Fig. 4).

The filtration coefficient was measured in these experiments by determining the filtration rate at different capillary pressures when both perfusate and superfusate contained identical concentrations of sucrose. The results of such an experiment where sucrose was present in the perfusate at a concentration of 30 m-mole.$l.^{-1}$ are shown in Fig. 4. In the three completed experiments, the average reflexion coefficient for sucrose was 0·100, i.e. approximately the same as its value determined when sucrose was added to the superfusate.

DISCUSSION

Our results show no significant relationship between σ and molecular size, a finding which we believe implies that water is able to cross capillary walls by a route unavailable to small hydrophilic molecules.

If water and solutes are able to cross a membrane through the same pores and by no other route, the absence of a correlation between σ and the molecular size of the solutes would be observed when the membrane pores were large in comparison with the test molecules (when the values of σ would all be close to zero) or when the membrane was semipermeable to all test solutes ($\sigma = 1$). The inconsistency between this prediction and our findings is demonstrated most clearly by our measurements of σ for more than one solute on the same capillary. The results of capillary *E* from Table 2 are compared, in Fig. 5, with theoretical curves consistent with the equations of Curry (1974) and Anderson & Malone (1974). A curve drawn for a pore radius of 1·1 or 1·2 nm fits the data for sodium chloride and urea but is inconsistent with the data for sucrose which is fitted by a theoretical curve for a pore radius of 2·4 nm. Since all the measurements presented in Fig. 5 were made on the same capillary, we conclude that our data are entirely incompatible with the model of an otherwise impermeable capillary wall penetrated by a single set of pores shared by water and hydrophilic solutes.

Our data conflict with the findings of Vargas & Johnson (1964) and Taylor & Gaar (1970) who described the expected correlation between

their estimates of σ and molecular size for the capillary walls of the rabbit heart and the dog lung respectively. Very recently Perl *et al.* (1975) estimated σ for small hydrophilic solutes in the dog lung and failed to show a correlation with molecular size. The measurements were so uncertain, however, that Perl *et al.* stated their findings were not necessarily in conflict with those of Vargas & Johnson (1964). Pappenheimer (1970 *b*)

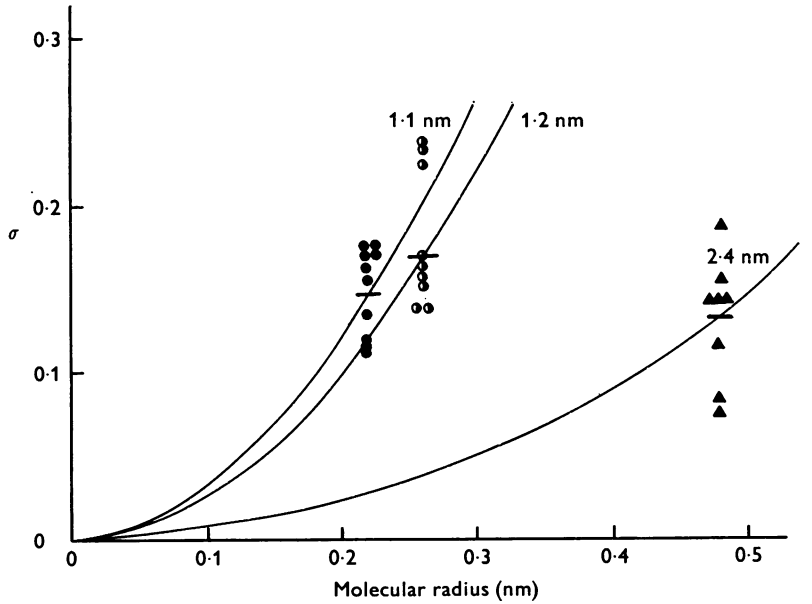

Fig. 5. The relationship between σ and the molecular radius of the test solutes in a single capillary. The data are those determined on capillary *E*, Table 2; the curves are theoretical curves for an otherwise impermeable membrane penetrated by cylindrical pores of different radii constructed from the equations of Curry (1974). Symbols: ●, NaCl; ◑, urea; ▲, sucrose.

criticized measurements of σ on perfused organs on the grounds that the transcapillary concentration difference (ΔC) responsible for the osmotic flow was estimated from the concentration of test solute in the inflowing blood or perfusate. He pointed out that during a single transit, ΔC could be considerably less than the inflow concentration for small rapidly equilibrating molecules. An over-estimate of ΔC gives rise to an underestimate of σ and since the error is largest for the smaller molecules, it could give rise to a spurious correlation between σ and molecular size. In our experiments on single capillaries, we attempted to minimize this error by controlling the composition of both the perfusate and the superfusate. It is possible, nevertheless, that we also over-estimated ΔC. Although it would mean that the values of σ in Tables 1 and 2 are under-

estimates, the error would not affect our principle conclusion for it would be expected to exaggerate rather than hide a dependence of σ upon molecular size.

Our results are, however, almost the prediction of a hypothesis suggested by Pappenheimer (1970 *a*) that, in osmotic transient studies, a large fraction of the water crossing the capillary wall travels by a pathway not available to small hydrophilic solutes. This exclusive water pathway is thought to be across the cell membranes and cytoplasm of the endothelial cells while both the hydrophilic solutes and water share a pathway through the intercellular region. Yudilevich & Alvarez (1967) provided evidence for at least 50 % of the water diffusing across the capillary walls (in the dog heart) by a route unavailable to Na^+ which they suggested lay through the bodies of endothelial cells. It has not been possible, hitherto, to estimate the contribution of this exclusive water pathway to net fluid flow across the capillary wall. Our results allow such an estimate to be made.

Transcellular and pore flow for water: estimation of their contributions to net fluid flow

Lifson (1970) followed up Pappenheimer's (1970 *a*, *b*) hypothesis with an expression for the average reflexion coefficient, $\bar{\sigma}$, in terms of the reflexion coefficient, σ_c, and the filtration coefficient, L_{pc}, for the 'transcellular' (exclusive water) pathway, and the reflexion coefficient, σ_p, and the filtration coefficient, L_{pp}, for the 'pore' pathway (channel shared with solute): i.e.

$$\bar{\sigma} = \frac{\sigma_c L_{pc} A_c + \sigma_p L_{pp} A_p}{L_{pc} A_c + L_{pp} A_p}, \tag{3}$$

where A_c and A_p are the fractions of the total area of capillary wall occupied by the 'transcellular' and 'pore' pathways. The average filtration coefficient, $\bar{L}_p$, is:

$$\bar{L}_p = L_{pc} A_c + L_{pp} A_p. \tag{4}$$

Since $\sigma_c = 1$ (by definition for an exclusive water pathway), eqn. (3) can be rearranged to yield an expression for $\bar{\sigma}_p$ and $\bar{L}_p$:

$$\bar{\sigma} = \frac{L_{pc} A_c}{\bar{L}_p}(1 - \sigma_p) + \sigma_p. \tag{5}$$

Eqn. (5) can be used to estimate L_{pc} in two ways. The value of σ_p should vary between different molecules with their molecular size in accordance with the theories of the osmotic reflexion coefficient of porous membranes (e.g. Bean, 1972; Curry, 1974; Anderson & Malone, 1974). Our finding that $\bar{\sigma}$ does not vary with molecular size suggests the σ_p is close to zero

for the solutes which we have investigated. If this is so, eqn. (5) approximates to:

$$\bar{\sigma} \simeq \frac{L_{p_c} A_c}{\bar{L}_p}. \tag{6}$$

The data in Tables 1 and 2 give an average value for $\bar{\sigma}$ of 0·115 and an average value for $\bar{L}_p$ of $1{\cdot}86 \times 10^{-3}$ μm.sec^{-1}.cm H_2O^{-1}. Substituting these values in eqn. (6) gives a value for $L_{p_c} A_c$ of $0{\cdot}214 \times 10^{-3}$ μm.sec^{-1}.cm/H_2O^{-1}.

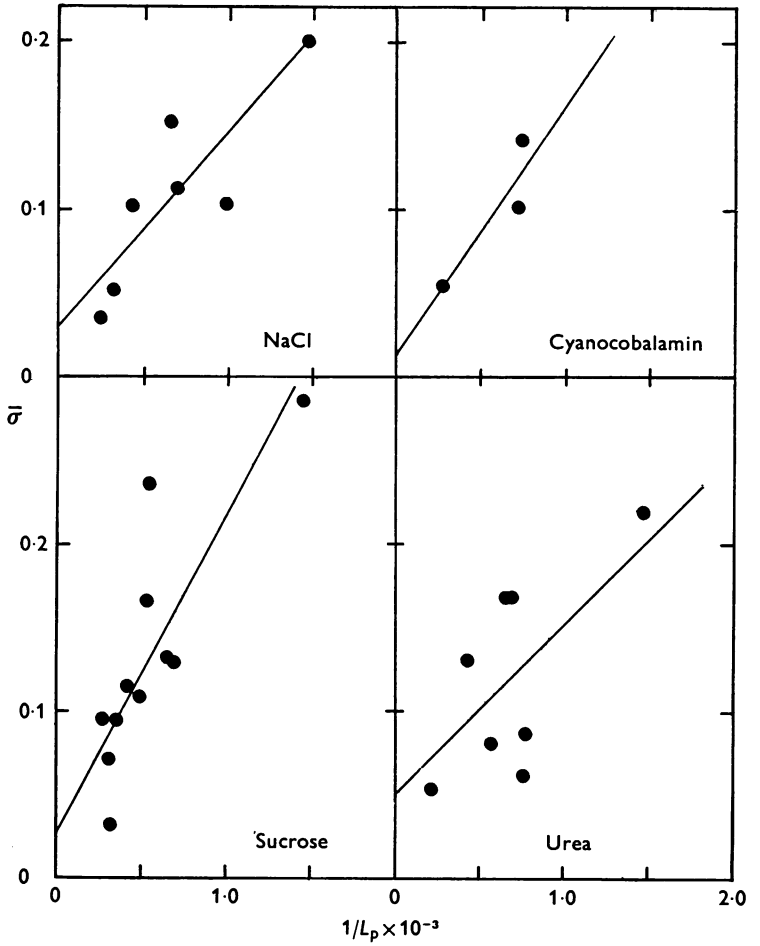

Fig. 6. The relationship between the average reflexion coefficient ($\bar{\sigma}$) of a capillary to a particular molecule and the reciprocal of the capillary's filtration coefficient. Each point represents a different capillary. The data from capillary *C*, in Table 2, have not been included owing to the uncertainty of the absolute value of the filtration coefficient.

Eqn. (5) can be used to calculate $L_{p_c}A_c$ in a rather different way if the parallel pathway hypothesis is used as a model for interpreting variations in permeability between different capillaries. If the variations in $\bar{L}_p$ and $\bar{\sigma}$ (for one particular solute) between different capillaries is the result of variations in the number of pores rather than variations in their equivalent pore radii, only $\bar{\sigma}$ and $\bar{L}_p$ will vary in eqn. (5) providing $A_c \gg A_p$. Thus values of $\bar{\sigma}$ for a particular solute should be linearly related to $1/\bar{L}_p$. The results of all experiments summarized in Tables 1 and 2 are examined from this point of view in Fig. 6. Although there is considerable scatter, a good correlation between $\bar{\sigma}$ and $1/\bar{L}_p$ is seen for the sodium chloride and sucrose data though the picture is less clear for urea. The intercepts on the ordinates of Fig. 6 are according to eqn. (5) the values of $\bar{\sigma}_p$ and although not statistically significant it is pleasing to see them indicating a positive value between 0·00 and 0·05. The slopes of the regression lines should have values equal to $L_{p_c}A_c(1-\sigma_p)$ and from the sucrose and sodium chloride data these yield values for $L_{p_c}A_c$ of $0{\cdot}114 \times 10^{-3}$ μm.sec^{-1}.cm H_2O^{-1}.

These calculations suggest that about 10% of the average filtration coefficient is represented by the exclusive water pathway (the cells), the remainder being accounted for by the shared pathway (the pore system). They also offer evidence for the hypothesis that variations in permeability properties between different capillaries arise from variations in the number rather than the dimensions of the 'pores'.

Let us now consider the relative flows through the exclusive water channel and through the shared channels under different conditions. If the solutions on either side of the capillary wall are of identical chemical composition but at different hydrostatic pressures, the fraction of fluid flowing through the exclusive water channel is $L_{p_c}A_c/\bar{L}_p$ and that flowing through the shared channel is $L_{p_p}A_p/\bar{L}_p$. Thus in capillaries of the frog mesentery, nine tenths of the flow resulting from a hydrostatic pressure gradient passes through the 'pores' and only one tenth traverses the 'cells'. It may be argued that the fraction of water travelling through the 'cells' is reduced still further by osmotic damping. The pure water leaving the exclusive water channel lowers the solute concentration here, giving rise to an osmotic pressure difference across the membrane which opposes the driving hydrostatic pressure difference. Osmotic damping may be important in limiting the flow through the exclusive water channels in some capillaries. Calculations suggest that in frog mesenteric capillaries, this kind of osmotic damping is significant only if the rate of diffusion of sodium chloride (the principal solute of the pericapillary fluid) through the interstitial space is more than one order of magnitude less than its value in free solution.

During an osmotic transient, however, the situation may be very different. Let us consider the conditions of an osmotic pressure difference in the absence of a difference in hydrostatic pressure. If J_v is the total flow of fluid across the capillary wall and J_{v_p} and J_{v_c} are the flows via the pore (shared channel) and cell (exclusive water channel) then since $\bar{\sigma}_c = 1$

$$J_v = J_{v_p} + J_{v_c} = L_{p_p} A_p \sigma_p \Delta\Pi + L_{p_c} A_c \Delta\Pi. \quad (7)$$

Our results suggest that $L_{p_c} A_c / \bar{L}_p = 0{\cdot}1$ and $L_{p_p} A_p / \bar{L}_p = 0{\cdot}9$. Therefore

$$J_{v_p} + J_{v_c} = 0{\cdot}9 \bar{L}_p \sigma_p \Delta\Pi + 0{\cdot}1 \bar{L}_p \Delta\Pi$$

or

$$\frac{J_{v_p}}{J_{v_c}} = 9{\cdot}0 \sigma_p. \quad (8)$$

Thus more water crosses by the shared channel during an osmotic transient only when $\sigma_p > 0{\cdot}11$. Our data suggest that for the small hydrophilic molecules σ_p lies between 0·00 and 0·05. When $\sigma_p = 0{\cdot}05$, 31 % of the water crosses by the shared channel and when $\sigma_p = 0{\cdot}01$ only 9 % crosses by this route.

Analysis of the osmotic transients

In Fig. 3 it is seen that when the test solute is present in the superfusate, the initial rate of filtration, measured immediately after occlusion, falls rapidly to about the control value as test solute equilibrates across the capillary wall. In the early stages of the investigation it was hoped that it would be possible to estimate the permeability of the capillary wall to solute from these changing osmotic flows. Later it was appreciated this would not be possible, for in our experiments solute equilibration was influenced by solvent drag and osmotic damping as well as by solute permeability.

Solvent drag would occur through the shared channel and since net flow and net solute movement were in opposite directions, it would be expected to lengthen the equilibration time. In the absence of accurate values of σ_p and J_{v_p} it is impossible to estimate accurately the contribution of solvent drag.

Osmotic damping would be expected to reduce the equilibration time. The concentration of test solute within the capillary would be raised not only by the entry of test solute from the pericapillary fluid but also by the extraction of water from the capillary via the exclusive water channel. When sodium chloride is the test solute, osmotic damping shortens the equilibration time in a simple fashion by concentrating the capillary contents because sodium chloride is the principle solute of both perfusate and superfusate. When other test solutes are used, osmotic damping may

bring the difference in osmotic pressure across the capillary wall to zero before diffusion has eliminated the concentration difference of urea or sucrose. Under these circumstances the filtration rate may be expected to fall below the control level, a prediction we have regularly seen fulfilled (Fig. 3).

Conclusions

Our results suggest that the permeability properties of frog mesenteric capillaries to water and hydrophilic solutes may be represented by a semipermeable membrane penetrated by a small number of pores (5·0–10·0 nm radius). In most circumstances net fluid movements occur via the pore system but large flows can occur across the semipermeable membrane when there is a concentration difference for small molecules between the blood and the tissues. It is imagined that the endothelial cells represent the semipermeable membrane and the pores are located at regions within the intercellular junction but alternative models exist and another attractive possibility is that the pores and the semipermeable membrane are represented by the open and tight regions of the intercellular junction.

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